## Supplemental Information

## Supplemental Experimental Procedures

### Mice

All experimental procedures were performed under approval by Washington University's Animal Studies Committee. Eight to twelve week old male C57BL/6J, BALB/cJ, BALB/cBYJ (*Acads*-deficient), *Foxo1*-floxed, and *Foxo3*-floxed mice were purchased from Jackson Laboratories and bred in house (stock number 000664, 000651, 001026, 024756, and 024668 respectively). All experiments used littermate controls. Knock-in mice expressing a fusion protein between Cdc25A and click beetle red luciferase in the endogenous *Cdc25a* locus were described previously (Sun et al., 2015). Mice overexpressing the S1 subunit of pertussis toxin under the control of the ROSA26 locus (ROSA26PTX) were described previously (Regard et al., 2007).

### Mouse Treatments

Mice were treated *ad libitum* with an antibiotics cocktail (VNAM) including vancomycin (0.5mg/ml), neomycin (1mg/ml), ampicillin (1mg/ml) and metronidazole (1mg/ml) (all Sigma) with Koolaid (20g/L), or metronidazole alone (1mg/ml). For DSS experiments, mice received 2.5% DSS in drinking water *ad libitum* for 7 days and were sacrificed on day 8. Colorectal injuries were created using the endoscopic-guided biopsy system as previously described (Seno et al., 2009), and mice were sacrificed on day 4. Mice received intra-rectal administration of sodium chloride or sodium butyrate (150 µmoles; twice a day) from day 5-7 (for DSS experiments) or day 1-3 (biopsy injury). To inhibit Foxo, mice were treated with the cell permeable small molecule Foxo family inhibitor (AS1842856; 0.1 µmoles) (Millipore) also from day 5-7. Transverse colon was examined for all analyses.

### Primary intestinal epithelial cell culture

Primary colonic epithelial stem cells were isolated, grown, passaged, and maintained as three-dimensional spheroid cultures in Matrigel (BD Biosciences) as described previously (Miyoshi et al., 2012; Miyoshi and Stappenbeck, 2013). Cells were maintained as enriched for stem/progenitor cells in 50% L-WRN conditioned media. For colonocyte differentiation, culture media was switched to differentiation media 24 hours after passage. Differentiation media contained Advanced DMEM/F12 (Invitrogen), Penicillin/Streptomycin (0.5mg/ml), L-glutamine (2mM), Y-27632 (10µM; R&D Systems), EGF (50ng/ml) and L-161982 (10µM Tocris). All experiments using *in vitro* differentiated colonocytes were performed 36 hours after the addition of differentiation media.

### Fecal Transplantation

Fecal samples were collected from BALB/cJ and BALB/cBYJ mice, re-suspended in sterile PBS and mixed together in anaerobic chamber. Mice were orally administered 100µL of the fecal mixture 14 days prior to DSS treatment.

### Metabolic assays

Trypsinized colonic spheroids were embedded in 2.5µL of Matrigel in XF96 cell culture plates and cultured in 50% conditioned media. For colonocytes, the media was changed to differentiation media the next day. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using Seahorse XF96e analyzer (Seahorse Bioscience). To measure basal metabolism, analysis was performed in XF Assay Medium (Seahorse Bioscience) supplemented with 2mM L-glutamine, 10mM glucose, 1mM pyruvate, 10% FBS. To measure butyrate oxidation, analysis was performed in low glucose FAO buffer (110mM NaCl, 4.7mM KCl, 2mM $MgSO_4$, 1.2mM $Na_2HPO_4 \cdot 7H_2O$, 2.5mM glucose) under basal conditions and following the addition of 2-Deoxy-D-glucose (3mM; Sigma), sodium chloride or sodium butyrate, and Rotenone (100nM; Sigma) and Antimycin A (1µM; Sigma). In separate experiments, analysis was performed in low glucose FAO buffer under basal conditions and following the addition of sodium chloride or short chain fatty acids (sodium acetate, sodium propionate, sodium butyrate), Oligomycin (2µM; Sigma), Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP; 3µM; Sigma), Rotenone (100nM; Sigma) and Antimycin A (1µM; Sigma).

### Immunostaining of colonic tissues and cells

Colonic tissues or *in vitro* spheroids were fixed in 4% paraformaldehyde in PBS for 16 hours. Tissues/cells were further processed for paraffin embedding or OCT embedding as previously described (Miyoshi et al., 2012). For immunofluorescence analyses, the sections were boiled in Trilogy solution (Cell Marque) for 20 minutes, rinsed in PBS, blocked with 10mg/ml bovine serum albumin/0.1% Triton-X 100 for 30 minutes, and incubated with primary antibody at 4°C overnight. Primary antibodies included anti-CD44 (R&D), Ki67 (clone TEC-3; DAKO), β-catenin

(BD Transduction Laboratories), cleaved caspase 3 (Cell Sigmaling), Aquaporin 8 (Bioss), Car4 (gift from William Sly), Acads (Abcam), F-actin. For immunofluorescence, fluorophore-conjugated (Alexa Fluor 488, Alexa Fluor 594, Alexa 647) secondary antibodies were used to visualize the signal. Nuclei were visualized by bis-benzimide/Hoechst (Invitrogen). Images were captured with a Zeiss Axiovert 200 microscope with an Axiocam MRM digital camera. For immunohistochemistry, biotinylated secondary antibodies (Vector Laboratories) were used, followed by streptavidin-HRP incubation (Vectastain ELITE ABC kit; Vector Laboratories). Staining was visualized by DAB (3, 3'-diaminobenzidine) peroxidase substrate (Vector Laboratories). Goblet cells were visualized by Alcian Blue staining. For *in vivo* experiments, Ki67-positive nuclei were quantified per crypt or per length of the tissue. For *in vitro* experiments, cleaved caspase3 staining was quantified by counting the number of cleaved caspase 3-positive cells per individual epithelial spheroid.

**Alkaline phosphatase (AP) staining**

Rehydrated paraffin sections were incubated with 5-Bromo-4-chloro-3-indolyl phosphate (BCIP; 0.17ng/ml; Roche) and Nitro blue tetrazolium chloride (0.33mg/ml; Roche) in buffer (100 mM Tris–Cl, 100 mM NaCl, 5 mM MgCl2, pH 9.5). Labeling reactions were performed at room temperature in the dark. Signal development was viewed briefly at regular intervals using light microscopy. Control reactions containing AP inhibitors (5mM levamisol and high heat) were used.

**Zebrafish experiments**

AB* WT embryos were collected from natural spawnings and maintained in egg water (2.4 g Instant Ocean in 8L of $ddH_20$, pH 6.5). On day 5 post fertilization, larvae were treated with sodium chloride, sodium butyrate or Trichostatin (TSA) as indicated. At the end of the treatment, larvae were sacrificed by Tricaine (3-aminobenzoic acidethylester) overdose and fixed in 4% paraformaldehyde in PBS for 16 hours. Larvae were embedded in agar to orient them in paraffin blocks. Thirty consecutive 5µm sections were cut starting at intestinal-esophageal junction. EdU and TUNEL staining were performed using the Click-iT EdU Imaging kit and the Click-iT TUNEL Assay for In Situ Apoptosis Detection kit (Life Technologies). EdU and TUNEL-positive nuclei were counted exclusively in the epithelial compartment, and the counts were normalized by the diameter of the intestine (arbitrary unit) measured using Image J software.

**Colonic Stem/Progenitor vs surface colonocyte layer enrichment**

Isoflurane-anesthetized mice were cut open in the abdomen, and colon was flushed with pre-warmed (37°C) 1X Dulbecco's Phosphate-Buffered Saline (DPBS; Life Technologies). Thoracic cavity was cut open, and 30mM EDTA (in Hanks Balanced Salt Solution (HBSS, Calcium/Magnesium free; Life Technologies) pH 7.4, 37°C solution was perfused from left ventricle at a constant flow rate (total 30ml in 3 minutes). The inferior vena cava was cut immediately after starting the perfusion. Colon was removed, cut in 2-3 pieces and flipped inside-out. Colon tissue was incubated in 10ml of 1mM EDTA/1mM Dithiothreitol (DTT) solution for 10 minutes at room temperature, followed by a second incubation in 10ml of 1mM EDTA/HBSS solution for 10 minutes at 37°C. Tissue was transferred to 2ml tubes containing pre-warmed 1mM EDTA/HBSS solution, shaken with mini a bead-beater (Biospec Products) at 2500 oscillations per minute for 20 seconds. After shaking, the colonic tissue fragments were removed (lamina propria and submucosa) and crypts were settled on ice, and washed with ice-cold HBSS three times. To obtain a single-cell suspension, crypts were transferred to 30ml of Dispase solution in HBSS (1.2U/ml Dispase, 25µg/ml DNase, 0.5mM $MgCl_2$; Sigma-Aldrich) and incubated at 37°C for 3 minutes, followed by gentle pipetting. The cell suspension was filtered through a 70µm filter and centrifuged at 1000 rpm for 10 minutes at 4°C. Cells were resuspended in sorting buffer (PBS/2mM EDTA/0.5% BSA) incubated with magnetic beads (Dynabeads, Life Technologies) labeled with anti-CD44 antibody (BD Biosciences) and magnetically separated into CD44-positive and -negative fractions.

**Mass spectrometry analyses**

Cell pellets were dried in a speedvac to remove residual water, then the pellets were extracted with 100µL 90% acetonitrile containing 1% formic acid and 10µM d7-butyric acid (D-171, CDN Isotopes) as an internal standard to normalize the data. The pellets and extraction solvent were vortexed for 15 minutes to completely extract the cells. The samples were centrifuged to remove precipitates. One microliter was injected onto a 0.5 x 150 mm BioBasic AX column (Thermo Scientific) on an Agilent 1200 Capillary LC system attached to a Q-Exactive mass spectrometer (Thermo-fisher Scientific). The gradient conditions were: hold at 100%B for 4 minutes followed by a linear gradient down to 20%B over 16 minutes, a hold at 20%B for two minutes followed by a ramp back to 100%B over one minute and a re-equilibration time of seven minutes. The solvents used were 95% acetonitrile with 10mM ammonium bicarbonate (B) and 10mM ammonium bicarbonate (A). Mass spectrometric data were collected in polarity profile mode with a scan range of *m/z* 70-1000 and with SIM scans of *m/z* 77-97, 96.5-105.5 (both negative mode for butyrate and acetoacetate detection, respectively) and *m/z* 800-870 for acetyl-CoA. The data were analyzed with QuanBrowser (Thermo-Fisher Scientific); all data were normalized based on the area of the internal standard peak (d7-butyric acid). Luminal contents were flushed from the colon of untreated C57BL/6J and snap frozen for analysis by mass spectrometry. Samples were diluted 1:10 water containing the following deuterated standards 10µM d7-butyric acid, d7-m-toluic acid, d2-5-hydoxyindole, d4-tyramine, d3-pyridoxal; d4 deoxycholic acid, d5 indole 3-acetamide; 100µM d8-spermidine (CDN Isotopes) by vortexing for 15 minutes. All samples were centrifuged to remove particulates and filtered prior to LC-MS. One microliter was injected onto a 0.5 x 100 mm PLRPS column (Higgins Analytical) on an Agilent 1200 Capillary LC system attached to a Q-Exactive mass spectrometer (Thermo-fisher Scientific). The gradient conditions were: hold at 0%B for four minutes followed by a linear gradient to 30%B over four minutes, then up to 100% B in three minutes a hold at 100%B for four minutes followed by a ramp back to 0%B over two minutes and a re-equilibration time of thirteen minutes. The solvents used were 95% acetonitrile with 10mM ammonium bicarbonate (B) and 10mM ammonium bicarbonate (A) for butyric acid, m-toluic acid, and deoxycholic acid analysis, and water (A) and acetonitrile (B) with 0.1% formic acid for pyridoxal, tyramine, spermidine, indole 3-acetamide and hydroxyindole. Mass spectrometric data were collected in negative profile mode with a scan range of *m/z* 80-400 and positive profile mode with a scan range of *m/z* 120-220. The data were analyzed with QuanBrowser (Thermo-Fisher Scientific); concentrations of metabolites in the samples were calculated point-to-point based on the area of the internal standard peaks.

**Isotope tracing experiments**

For *in vitro* isotope tracing analyses, cells were treated with 3mM $^{13}$C4-butyrate (Sigma) in FAO media supplemented with 2.5mM glucose. After three hour incubation, cells were harvested in Cell Recovery Solution (Corning) on ice for 30 minutes before cell pellets were snap frozen for mass spectrometry analysis. For *in vivo* isotope tracing analyses, mice received VNAM for two weeks, followed by intrarectal administration of $^{13}$C4-butyrate (150µmoles) 24 hour and 1 hour prior to sacrifice. Colonic epithelial cells were harvested and further enriched for surface colonocytes, CD44-negative crypt colonocytes, and CD44-positive stem/progenitor cells by magnetic bead sorting using the CD44 antibody. Once cells were harvested, a small fraction of the cell suspension was collected for DNA quantification for normalization, and the remaining fraction was snap frozen for mass spectrometry analysis.

**RNA isolation and quantitative real-time PCR**

RNA was extracted from *in vitro* mouse epithelial cultures using the Nucleospin RNA isolation kit (Clontech) and following manufacturer's instructions. cDNA was generated using SuperScript III (Invitrogen). Gene expression was quantified using SYBR Green reagents (Clontech) on an Eppendorf Mastercycler (Eppendorf). Delta Ct values were calculated relative to the housekeeping gene *Gapdh* for mRNA expression. For the bacterial genes delta Ct values were calculated relative to the control gene *rpoB* (Nava et al., 2011) for quantification of colonization by Lachnospiraceae/Ruminococcaceae (Nava et al., 2011), or Clostridia Cluster XIVa (Matsuki et al., 2004), and butyryl-Coenzyme A (CoA) CoA transferase levels (BCoAt) (Louis and Flint, 2007).

**GO term analysis**

GO terms (Biological Processes) for stem cells versus colonocyte-associated genes (>2-fold increase) from the microarrays were classified using Panther Classification System (Geneontology). Genes sets associated with primary metabolic processes were further classified into various more specific metabolic processes.

**Immunoblotting**

Colonic epithelial stem cells were harvested using Cell Recovery Solution (BD Biosciences), pelleted, and further processed for subcellular fractionation using Subcellular Protein Fractionation Kit (ThermoFisher). The nuclear fraction was subjected to electrophoresis using Any-KD Mini-Protean gels (Bio-Rad) and transferred onto nitrocellulose membrane (Bio-Rad). Membranes were blocked with Blocking One (Nacalai USA) and incubated with primary antibodies (anti-Histone H3; Cell Signaling, anti-acetylated H3K9; Cell Signaling, anti-acetylated H3K27; Abcam, anti-Foxo3; Millipore) in 5% BSA in TBS (1% Tween-20) buffer overnight at 4°C. Membranes were then incubated with HRP-conjugated secondary antibodies (Bio-Rad) for 1 hour at room temperature. Signal was detected using the SuperSignal West Dura chemiluminescence kit (Thermo Fisher Scientific).

**EdU Click-iT cell cycle flow cytometry assay**
Cells were incubated with 10µM EdU for 2 hours. Epithelial spheroids were isolated from 3D Matrigel culture in PBS-EDTA. A single cell suspension was obtained by trypsinization. The EdU Click-iT Flow Cytometry Assay kit (Invitrogen) was then used to determine the percentage of S-phase cells according to the manufacturer's instructions. Flow cytometry data was analyzed using FloJo V10 software.

**Microarray**
RNA was extracted using Nucleospin RNA isolation kit (Clontech). Microarray analysis was performed on 4x44K mouse whole genome microarrays (Agilent) as previously described (Cadwell et al., 2010). Hierarchical clustering, heatmap expression analysis, and statistical analysis were performed using Partek software.

**Histone deacetylase (HDAC) activity assay**
The level of HDAC activity in colonic epithelial stem/progenitor cells at baseline as well as the ability of butyrate to inhibit this stem cell HDAC activity was quantified using the enzyme-based fluorometric Histone Deacetylase (HDAC) Activity Assay Kit (Abcam) according to manufacturer's instructions.

**Colony forming efficiency assay**
Colonic epithelial spheroids were removed from Matrigel with PBS-EDTA and trypanized for 10 minutes at 37°C with vigorous pipetting to break up epithelial fragments into single cells. This was monitored by light microscopy. Cells were washed, passed through a 40µm filter, counted, and resuspended in Matrigel at a concentration of 1 cell per 5µl Matrigel. Each well of a 96 well plate received 5µl Matrigel. After the Matrigel had solidified 100µl of 50% L-WRN media was added to each well, and every well from every 96 well plate was examined under the light microscope at high magnification to identify those wells that contained only one epithelial cell. Wells which contained more than 1 cell or no cells were eliminated. Cells were then incubated for 5 days (with media changed every 2 days) after which time the number of single cells that formed a spheroid colony was quantified (colony forming efficiency %).

**Screening and luciferase proliferation assay**
Colonic spheroids were generated from Cdc25A-luciferase reporter mice. Upon addition of D-luciferen luminescence from Cdc25A-luciferase expressing cells provided a direct readout of mitotic activity. Stem/progenitor cells from the spheroids generated from these mice were trypsanized for 3 minutes at 37°C with vigorous pipetting to break up epithelial spheroids into small homogenous fragments. This was monitored by light microscopy. Cells were washed, passed through a 40µm filter to remove any larger fragments, and plated out in Matrigel in 96 well plates ready for screening. Cells were grown in either 50% L-WRN (high Wnt) for 48 hours or 50% L-WRN for 24 hours followed by 0% L-WRN (no Wnt) for the final 24 hours. Metabolites or PAMPs were added 24 hours after plating and luminescence readings (1 second) were taken at 0, 4, 16, 20, and 24 hours after addition of metabolites/PAMPs using an Envision 2101 plate reader. In the initial screen of the impact of metabolites on stem/progenitor cell proliferation the metabolites were added at a concentration of 1mM this was then followed by dose curve analysis. This luciferase proliferation assay was also used to measure the effect of various selective inhibitors including those targeting Gq signaling U73122 (Tocris), Gi signaling (Pertussis toxin from *Bordatella pertussis*; Sigma), HDAC1 (Parthenolide; Sigma), HDAC3 (RGFP966; Selleckchem), HDAC4/5 (LMK 235; Tocris), HDAC6 (Tubastatin A hydrochloride; Santa Cruz), HDAC8 (PCI-34051; Selleckchem), RARβ (LE 135; Santa Cruz), PI3-kinase (Wortmannin; Invivogen), Foxo1/3/4 (AS1842856; Millipore), TGFβIR (SB-431542; Tocris), and p300/CBP (C646; Sigma). A custom designed antagomir to inhibit microRNA-34c (Sigma) had the following sequence
5'mG.*.mC.*.mA.mA.mU.mC.mA.mG.mC.mU.mA.mA.mC.mU.mA.mC.mA.mC.mU.mG.*.mC.*.mC.*.mU.*.3'-Chol, where "m" represents 2'-OMe-modified phosphoramidites, "*" represents phosphorothioate linkages, and "-Chol" was hydroxyprolinol-linked cholesterol to allow permeation of cell membranes.

**FAIRE-seq and ChIP-seq**
FAIRE-Seq: Cells were fixed with 1% formaldehyde in culture medium for 10 min at room temperature followed by quenching with 0.125 M glycine for 5 min. Crosslinked chromatin was lysed for 10 minutes sequentially in L1 buffer (50mM Hepes KOH pH 7.5, 140mM NaCl, 1mM EDTA pH 8.0, 10% glycerol, 5% NP40 and 0.25% Triton X-100) followed by L2 buffer (200mM NaCl, 1mM EDTA pH 8.0, 0.5mM EGTA pH 8.0, 1M and Tris pH 8.0). The pellets were resuspended in 300ul of buffer L3 (1mM EDTA pH 8.0, 0.5mM EGTA pH 8.0, 1M Tris pH 8.0,

100mM NaCl, 0.1% sodium-deoxycholate and 5mg/ml N-lauroyl sarcosine) and subjected to sonication using a Bioruptor to an average size of 500bp. The soluble DNA fraction was isolated by performing two consecutive phenol:chloroform:isoamylalcohol (25:24:1, pH 8.0) extractions followed by a chloroform-isoamyl alcohol (24:1) extraction. Precipitated DNA was resuspended in 10mM Tris-HCl (pH 7.4) and incubated with 10µg of RNase A for 1hour. Genomic DNA was isolated using QIAquick PCR purification column. ChIP-Seq: Cells were fixed with 1% formaldehyde in culture medium for 10 min at room temperature followed by quenching with 0.125M glycine for 5 min. The cells were washed with ice-cold PBS and lysed in SDS lysis buffer (1% SDS, 10mM EDTA, 50mM Tris pH8.0) for 20 min on ice. The crosslinked chromatin was sheared to an average size of less than 300 bp using a BiorupterTM sonicator (Diagenode). 5µg of antibody was incubated with Dynabeads Protein A beads (Invitrogen) at 4°C for 4 hours. Sonicated lysates were subjected to immunopreciptation overnight at 4°C with acetylated H3K27 antibody (Abcam; ab4729) or Isotype control IgG conjugated to Dynabeads (Invitrogen, USA) coated with Protein-A. After incubation, the immune complexes were collected by centrifugation and washed with the following buffers each for 3 min at 4°C; low salt (150mM NaCl, 50mM Tris pH8.0, 0.1% SDS), high-salt buffer (500mM NaCl, 50mM Tris pH 8.0, 0.1% SDS), LiCl buffer (250mM LiCl, 50mM Tris pH 8.0, 0.5% sodium deoxycholate, 1% NP40) and TE (10mM Tris pH8.0, 1mM EDTA). The protein-DNA complexes were eluted from the beads in 250 µl elution buffer (1% SDS, 100mM NaHCO3) at 65°C overnight followed by the addition of proteinase K to 500µg/ml and incubated at 56°C for 1 hour. Genomic DNA was isolated using QIAquick PCR purification column (Qiagen). At least 5ng of ChIP or input DNA was used for library preparation according to standard Illumina ChIP-seq protocol. After end-repair and indexed adapter ligation, fragments were size-selected using Agencourt AMPure XP beads (Beckman Coulter) prior to amplification. The size-selected and amplified fragment libraries were verified on a 2100 Bioanalyzer (Agilent) prior to being pooled (7-10 per lane) and sequenced. Sequencing tags from FAIRE- and ChIP-Seq were aligned to the reference genome (mouse MM10) with Novoalign. MACS was used to identify FAIRE or ChIP peaks by comparison of matched treatment to input samples using default settings. Predictive algorithms (Bailey et al., 2009; Zambelli et al., 2009) were used to identify the common DNA binding motifs enriched in both the upstream promoter regions identified in the ChIP-seq, and the promoter regions of genes with altered expression after butyrate treatment.

**Electron microscopy**

For transmission electron microscopy, colon spheroids were cultured in 50% L-WRN for 48 hours or changed to differentiation media (recipe above) after 24 hours for a final 36 hours. The spheroid-Matrigel mixtures were scraped out of the tissue culture plate wells with a pipette tip in 1 mL of PBS and centrifuged for 5 min at 1000 rpm to pellet the cells. The PBS was removed and the spheroids were fixed in 2% paraformaldehyde/2.5% glutaraldehyde in 100 mM cacodylate buffer, pH 7.2 for 2 hour at room temperature. Samples were washed in cacodylate buffer and postfixed in 1% osmium tetroxide (Polysciences Inc.) for 1 hour. Samples were then rinsed extensively in $dH_20$ prior to en bloc staining with 1% aqueous uranyl acetate (Ted Pella Inc) for 1 hour. Following several rinses in $dH_20$, samples were dehydrated in a graded series of ethanol and embedded in Eponate 12 resin (Ted Pella Inc.). Sections of 95 nm were cut with a Leica Ultracut UCT ultramicrotome (Leica Microsystems Inc), stained with uranyl acetate and lead citrate, and viewed on a JEOL 1200 EX transmission electron microscope (JEOL USA Inc) equipped with an AMT 8 megapixel digital camera (Advanced Microscopy Techniques).

**TAT-CRE recombinase treatment of spheroids**

Colonic epithelial spheroids generated from floxed mice were removed from Matrigel with PBS-EDTA and trypanized for 5 minutes at 37°C with vigorous pipetting to break up epithelial spheroids into 1-3 cell fragments. This was monitored by light microscopy. Cells were washed and passed through a 40µm filter. Cells were then incubated in media with TAT-CRE recombinase (500µg/ml; Millipore) for 5 hours at 37°C. Cells were then re-suspended in Matrigel plated out and cultured for 2 days with 50% L-WRN media. Single spheroids were picked using an inverted microscope and plated out for expansion in fresh Matrigel in 24 well plates. After 2 days DNA from individual spheroids was genotyped and those with Cre excision of the LoxP sites were further expanded for *in vitro* assays. Individual epithelial spheroids with the floxed sites intact were also kept as controls for *in vitro* assays.

**Supernatant transfer experiment**

Colonic spheroid stem cells, colonocytes, or Matrigel only controls were incubated with media containing sodium butyrate (3mM) overnight at 37°C. The supernatants were then transferred to Cdc25A-luciferase expressing colonic stem cells and proliferation was monitored over 24 hours. In add back experiments the equivalent amount of butyrate that was depleted from the supernatant by incubation with colonocytes *in vitro* (as determined by mass spec analysis) was added back to the supernatant taken from colonocytes followed by exposure to Cdc25A-luciferase expressing stem cells.

***In vivo* histological measurement**

Atrophic crypts were defined as crypt remnants containing few to no dividing cells. Unhealed ulcer (DSS model) or wound (colon endoscopic biopsy model) area was determined by blinded quantification using macroscopic images of the colon taken with a whole mount microscope at 20x (Olympus SZX12). The percentage ulcerated area with hyperproliferation was determined by measuring the percentage of the ulcerated area that was directly surrounded by heaped (hyperproliferative) boundaries using blinded macroscopic images of the colon taken with a whole mount microscope at 20x.

**ChIP pull down and quantitative PCR**

Cells were treated and genomic DNA isolated as described above (see ChIP-seq) with the following modification. Anti-Foxo3 (5µg; Millipore; 07-702) or isotype IgG control (5µg) was incubated with Dynabeads Protein A beads (Invitrogen) at 4°C overnight. Sonicated cell lysates were subjected to immunoprecipitation overnight at 4°C with the antibody-conjugated dynabeads. DNA was analyzed by quantitative PCR using multiple primer sets designed around the promoter sites of the gene of interest.

| **Gene** | **Forward primer 5'-3'** | **Reverse primer 5'-3'** |
|---|---|---|
| Foxo3 mRNA | CGTTCCTGAAGGGAAGGAG | GCTTGGGCTCTTGCTCTCT |
| Gadd45b mRNA | CTGCCTCCTGGTCACGAA | TTGCCTCTGCTCTCTTCACA |
| Cdkn1a mRNA | AACATCTCAGGGCCGAAA | TGCGCTTGGAGTGATAGAAA |
| Cdkn1c mRNA | CAGGACGAGAATCAAGAGCA | GCTTGGCGAAGAAGTCGT |
| Ascl2 mRNA | TCTTGGGGCTTAAGGGCTGA | GTCAAGGTGTGCTTCCATGC |
| CD44 mRNA | GCATCGCGGTCAATAGTAGG | CACCGTTGATCACCAGCTT |
| Lgr5 mRNA | CATCTAGGCGCAGGGATTGA | GTCTCCTACATCGCCTCTGC |
| Aquaporin 8 mRNA | AAGTGTCCACCGCTATGTT | AGTCCGAATACTGGGCTCCT |
| Car4 mRNA | CGTCTTTCCCCTCAAGCAC | AATCTCATAGCACCAGCCTGA |
| Gadd45b ChIP DNA | GCCCTTTGTGCATCTACCAA | CAGAAGTGTCGGAGGCCTT |
| Cdkn1a ChIP DNA | ACGCTATAAGGAGGCAGCTC | ACTATTGTTCCCTGCCACGA |
| Cdkn1c ChIP DNA | CGACGTCGGTGAAGGTCC | GCCCTAATCAGTTGGTGCAG |
| rpoB | AACATCGGTTTGATCAAC | CGTTGCATGTTGGTACCCAT |
| Lachnospiraceae/Ruminococcaceae 16S | CGGTACCTGACTAAGAAGC | AGTTTYATTCTTGCGAACG |
| Clostridia cluster XIVa | AAATGACGGTACCTGACTAA | CTTTGAGTTTCATTCTTGCGAA |

| | | |
|---|---|---|
| Butyryl-Coenzyme A (CoA) CoA Transferase | GCIGAICATTTCACITGGAAYWSITGGCAYATG | CCTGCCTTTGCAATRTCIACRAANGC |
| Acads | CGTAGAGCTCTCGGTGTTCG | GACCAACTCCTTCTCGGCAA |

**Statistics**

GraphPad Prism software (version 6) was used to perform all statistical analyses unless otherwise specified.

**Supplemental References**


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